Available CD4 technology

Technology for which independent, peer-reviewed performance evaluation data is available:

Flow cytometry

- Dual platform
- Single platform bead-based technology on standard flow cytometer:
  - TruCount beads
  - FlowCount beads
  - Perfect Count
- Single platform dedicated CD4 flow systems:
  - FACSCount
  - Guava Easy CD4
  - Partec CyFlow Counter, Partec CyFlow SL_3

Manual technologies

- Cytospheres
- Dynabeads

Technology in use but for which no peer-reviewed independent performance evaluation data is available:

- PointCare NOW
- Guava Auto CD4
- CD4 select
- Sysmex pocH-100i

Evaluation of the Performance of CD4 Technologies

Accuracy

- No gold standard technology or internationally recognised reference preparation exists for CD4

- Methods for evaluation of performance
  - Correlation alone is insufficient
  - Bland-Altman plot alone (with or without “limits of agreement”) is insufficient

- Misclassification probabilities provide more clinically useful information about the test under evaluation
  - Upward misclassification around a treatment threshold may be most clinically important (leading to delay of start of ART or prophylactic treatment in some patients)
  - Downward misclassification may result in the decision to treat large numbers of additional patients who have CD4 counts above the guideline threshold when using the reference test

Precision

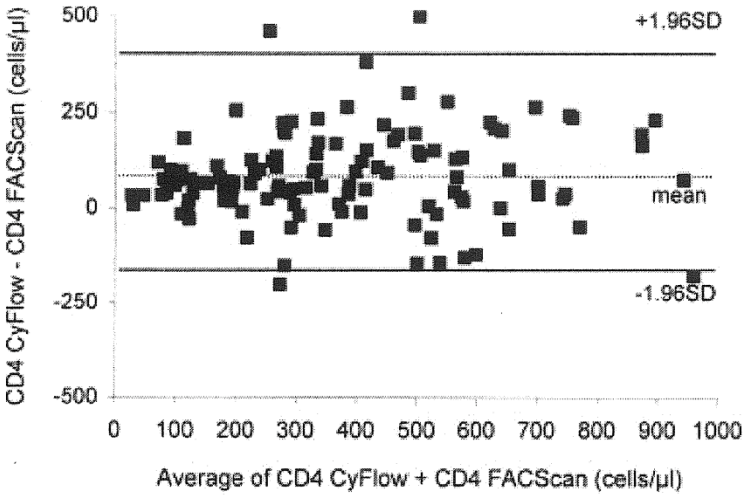
- Reproducibility of the new test when repeated on the same specimen. Includes within-run, between-run, between-operator, between-laboratory, usually measured as coefficient of variation (CV)

Systematic review of CD4 Technologies:

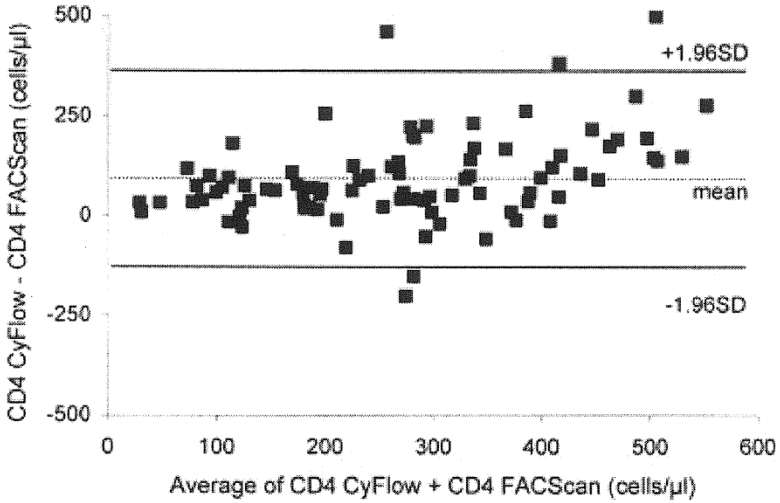
- What is already clear is that clinically relevant questions are difficult to answer from literature

- Studies often conclude that a method is an acceptable alternative to a reference method based on correlation alone, or based on a 'mean difference' between the two, which gives no indication of maximum differences seen (which may be large, despite a small mean difference), and which is often different at different levels of CD4, even within the clinically important range

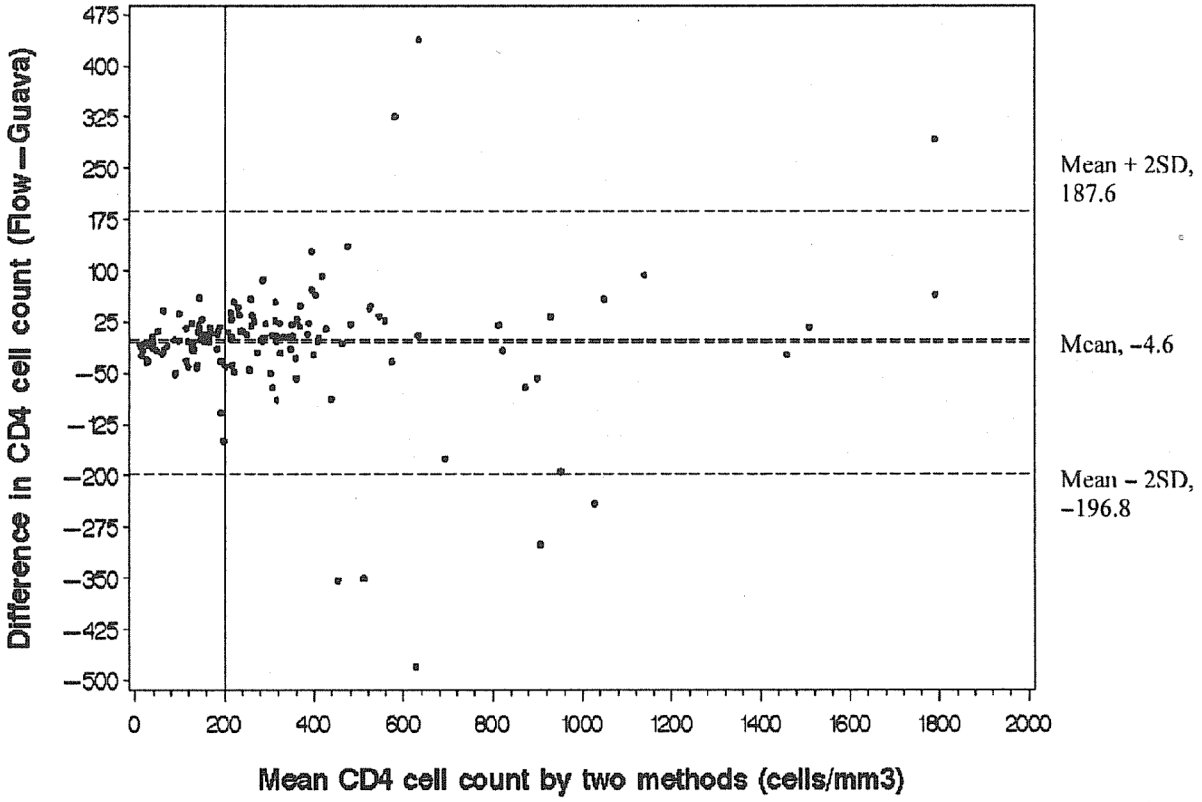
- Of 31 studies that fit the inclusion criteria, 15 gave data from which misclassification on either side of 200 can be calculated, and only 5 provided data which allowed calculation of misclassification on either side of 350



From Karcher et al 2006 Cytometry
Part B 70B:163-169



On correlation analysis, r=0.929
However, 29% of specimens with CD4<350 using FACScan misclassified as >350 when using CyFlow



Mean difference minimal (4 cells/µl).
But maximum differences large (-500 to + 400)

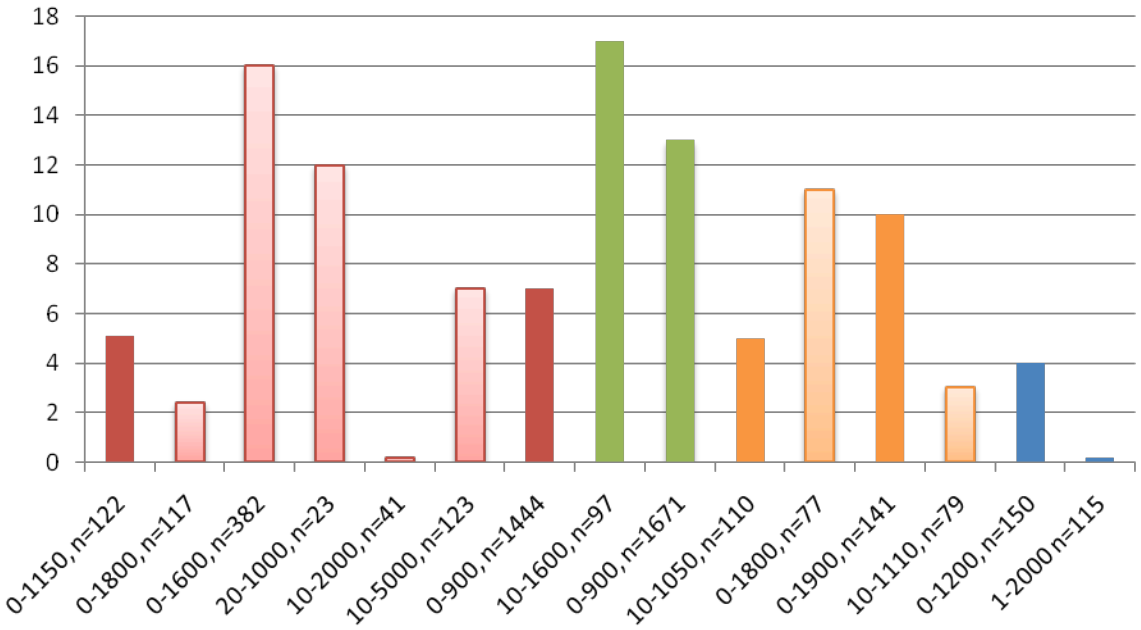
From Spacek et al. J Acquir Immune Defic Syndr Vol 41, 5, 2006

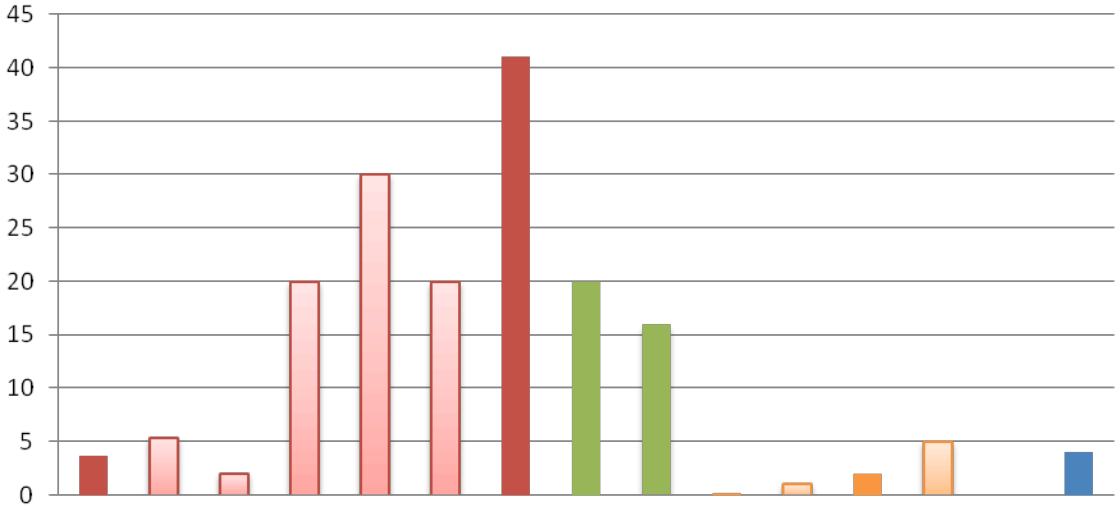
Cytospheres

Dynabeads

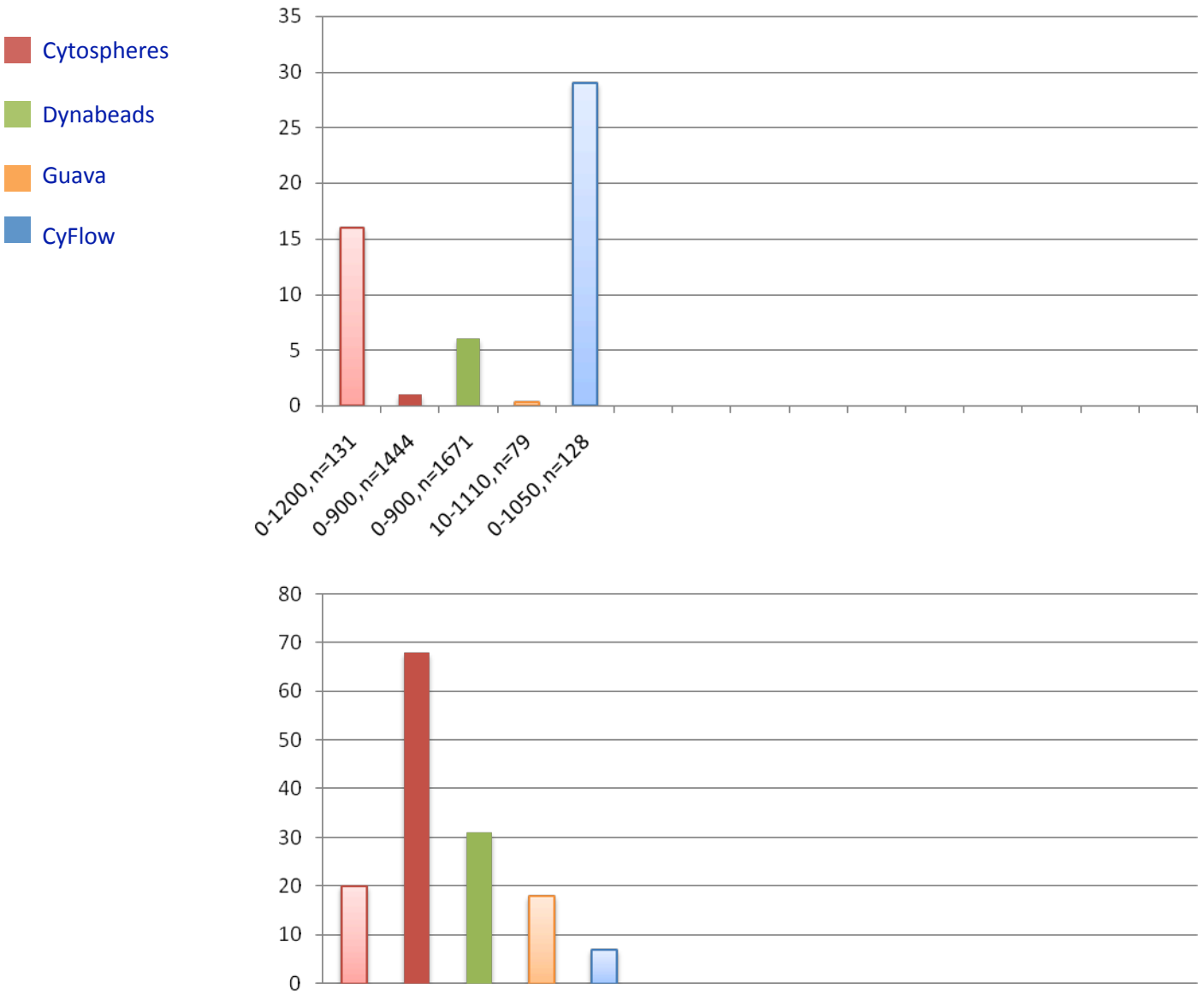
Guava

CyFlow





Misclassification up (upper figure) and down (lower figure), using a threshold of 200 cells/µl



Misclassification up (upper figure) and down (lower figure), using a threshold of 350 cells/µl.

- Both misclassification up and down are likely to be underestimates (particularly down) as none of the studies are restricted to the most clinically relevant range
- We cannot tell from the published papers the magnitude of the misclassification: are they mostly barely away from the threshold (e.g. 10 CD4 cells) or are they mostly far away (e.g. 100 CD4 cells)?
- A more pertinent question might be how many samples in the 150 - 250 range are being misclassified as having CD4 > 350, or how many samples in the 450-550 range are being misclassified as < 350
- But this is impossible to answer from the published literature (although authors likely to have primary data from which these could be calculated)

Precision

- Reproducibility on repeat testing of same sample by same method
- Important if following a patient's serial measurements
- Probability of misclassification is worse if precision is worse, although bias of 10% has more of an effect on misclassification than CV (measure of precision) of 10%

Method	Study	CD4 if given	No. of replicates per donor	Within-lab whole-process reproducibility on whole blood (CV%)	
				Index test	Flow cytometry
Cytospheres	1	200	?10	11	2.5
	2	Not given	?2	58	6
Dynabeads	3	Not given	2	8	8
	4	350	10	8	2

Given the limitations of available data, what can we say with any degree of confidence?

- There is variability associated with CD4 measurement, both physiological and technology-related, whichever technology is used
- Different technologies are associated with different performance characteristics, both in terms of misclassification and precision
- These characteristics, particularly misclassification, should be considered before choosing to implement a technology, but the data are not always available
- Participation in EQA programmes and access to QC reagents is essential

Hierarchy of current technologies based on performance levels

- Single platform flow cytometry > dual platform
  --doesn't rely on hematology analyzer, so less variability, especially with older blood specimens

- Dual platform >> Manual methods
  --lower misclassification probabilities, better precision, availability of EQA materials and programmes

- Difficult to place Guava and Cyflow in hierarchy
  --limited data on misclassification. Widely varying results with CyFlow in different papers, and wide variety of instruments and reagents make papers difficult to compare